



Murdoch
UNIVERSITY

MURDOCH RESEARCH REPOSITORY

This is the author's final version of the work, as accepted for publication following peer review but without the publisher's layout or pagination.

The definitive version is available at

<http://dx.doi.org/10.1016/j.pt.2013.10.006>

Thompson, R.C.A. and Lymbery, A.J. (2013) Let's not forget the thinkers. Trends in Parasitology, 29 (12). pp. 581-584.

<http://researchrepository.murdoch.edu.au/19455/>

Copyright: © 2013 Elsevier Ltd.

It is posted here for your personal use. No further distribution is permitted.

Let's not forget the thinkers

Q1 R.C. Andrew Thompson and Alan J. Lymbery

School of Veterinary and Life Sciences, Murdoch University, Murdoch WA, Australia

As 'omics' technologies become more accessible, enormous quantities of data are being generated about the genomes, proteomes, metabolomes etc. of an increasing number of parasites. We therefore need to think very carefully about how these resources will contribute to our basic understanding of parasitism, and beyond the 'knee-jerk' outcomes of new vaccines and therapeutics. The lasting legacy of the 'omics' era may lie in addressing the fundamental biological hypotheses generated by parasitologists 40–50 years ago when direct observational studies were a feature of parasitological research. We illustrate this with reference to the cestode parasite *Echinococcus* and the far-reaching questions posed by Desmond Smyth.

'Omics' data resources

As technology improves it is becoming easier and cheaper to sequence the complete genomes of biological organisms, and these appear with increasing frequency in the literature. This is certainly the case with parasitic organisms, particularly those of public health and veterinary significance, and the past few years have seen enormous advances in whole-genome sequencing, proteomic, and other 'omic' studies of protozoan and helminth parasites (e.g., [1–6]). These studies provide powerful resources, but we are concerned that the focus of their application is often too narrow. The drivers of this research are reiterated constantly as being the provision of data that can be exploited for identifying novel drug targets and vaccine candidates. However, are more fundamental issues and questions being overlooked? These are likely to be more far-reaching in terms of understanding developmental biology and host–parasite relationships, and thus will have greater impact in the long term on the development of 'novel' control strategies.

Utilizing 'omics' data

Such fundamental questions were often developed when parasitologists, and other scientists, had time to think, and their research funding was not driven by the current narrowed vision of granting bodies. Seminal thinkers such as Desmond Smyth must be turning in their graves!

Corresponding author: Thompson, R.C.A. (a.thompson@murdoch.edu.au).

Keywords: genomics; proteomics; models; developmental biology; *Echinococcus*; J.D. Smyth

1471-4922/\$ – see front matter

© 2013 Published by Elsevier Ltd. <http://dx.doi.org/10.1016/j.pt.2013.10.006>

The recent article by Tsai *et al.* [6] is an excellent example, in which the genomes of four disparate tapeworms are described and the main emphasis given to new drug targets as an outcome. There will always be a need for new treatments to combat tapeworm infections, and the search for new drug targets is important, but not an urgent priority; there are effective treatments for adult tapeworms and, although these are less effective against larval stages, drug treatment is not necessarily a viable option in this case. Of much more importance, we believe, is the light that comparative genomic and proteomic studies may shed on tapeworm development. From genome annotation, Tsai *et al.* [6] were able to make some interesting, although rather obvious, phylogenetic correlations of genomic and life history traits, such as a reduction in metabolic capacity and an increased ability to absorb nutrients associated with a parasitic lifestyle. Tantalizingly, the authors also refer to specialization, particularly regarding stem cells and tapeworm plasticity. This is a line of enquiry deserving much more study. It was originally discussed in the context of 'heterogeneous morphogenesis' in *Echinococcus* and new model systems by Smyth and colleagues in 1966 in *Nature* [7], and subsequently developed in a seminal paper on tapeworms as biological models in 1969 [8] in which tapeworm adaptations are discussed in depth.

Echinococcus as a model system

With respect to tapeworms, the situation is compounded by the fact that the original research on *in vitro* culture of *Echinococcus* and other tapeworms and their stem cells is not referred to in the recent literature on *in vitro* culture. As a consequence, fundamental questions and hypotheses, for which we at last have the appropriate technologies to address, are being overlooked.

For example, we already knew in the 1960s that tapeworms such as *Echinococcus* have tremendous developmental plasticity [8]. This is not a new observation, but with the sequence data we are now better placed to identify the mechanisms involved. Similarly, 'omics' data may provide the answers to questions of what governs host specificity [e.g., *Echinococcus granulosus* (broad range of intermediate hosts including humans) versus *Echinococcus equinus* (only equine intermediate hosts, not zoonotic); *E. granulosus* versus *Echinococcus multilocularis* (rodent intermediate hosts)] and molecular activities at the parasite–host interface, for example, the nature and function of rostellar gland nuclear secretions [9]. Unless we 'rediscover' the fundamental issues as laid down by earlier workers we may miss clues to guide data mining and thus short-circuit the search for truly novel control mechanisms.

Desmond Smyth pioneered *in vitro* cultivation techniques to support the development of the taeniid cestode *Echinococcus* in the laboratory. His detailed observations of the stages of development of *Echinococcus* induced *in vitro* and their inherent plasticity generated a wealth of information about developmental and physiological processes in cestodes and other parasites (Box 1). Most importantly, they provided the basis for generating a series of thought-provoking, seminal publications that raised numerous questions and hypotheses, most of which are not available online [10–21]. At the time, Smyth's work

challenged views on the simplicity of cytodifferentiation in platyhelminths. He also proposed that the *Echinococcus in vitro* system could be a model for both invertebrate and vertebrate studies, and this has still to be fully appreciated and exploited. This is especially timely given the recent rise of the field of evolutionary developmental biology, which is based to a large extent around the study of a limited number of model organisms [22]. Comparative evolutionary studies of development and cell differentiation in flatworms may yield great rewards. For example, the multipotential, stem cell-like nature of the postulated

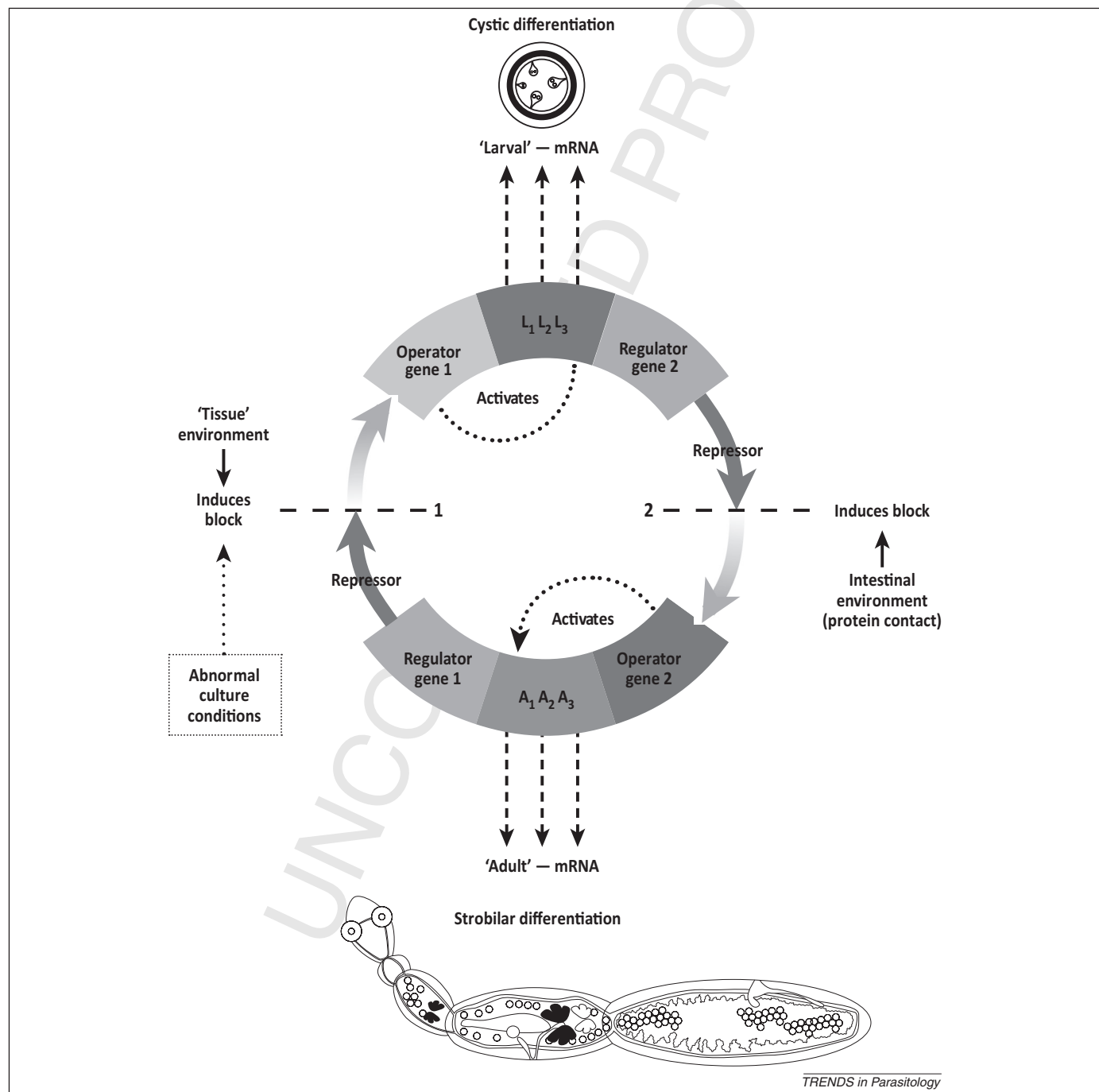


Figure 1. Control of differentiation in *Echinococcus*. Hypothetical control circuit, based on the Jacob–Monod model of gene action, for differentiation of *Echinococcus granulosa* into cystic (larval) or strobilar (adult) forms; much simplified, 'feedback' control omitted (see Figure 2). L_{1–3}, A_{1–3} larval and adult structural genes. Redrawn from Smyth (1969) [8].

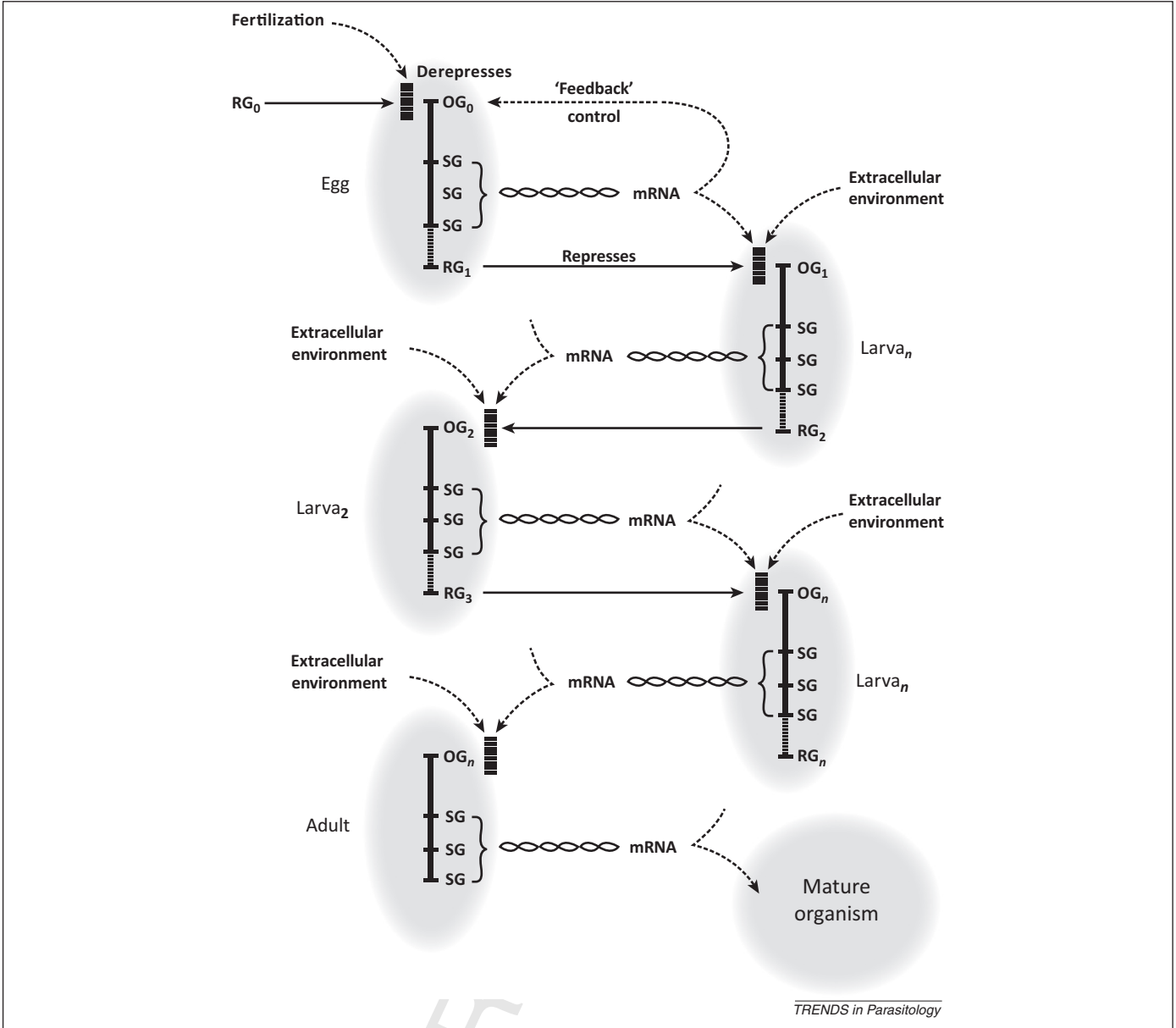


Figure 2. Regulatory factors in parasite life cycles. A hypothetical control circuit appropriate to the life cycles of parasites in general; based on the Jacob–Monod model of gene action. Abbreviations: OG, operator gene; RG, regulator gene; SG, structural gene. Redrawn from Smyth (1969) [8].

germinal cells of *Echinococcus*, which form part of the parasite’s ill-defined, syncytial ‘germinal layer’ in the metacystode and neck region of the adult [23,24] (reviewed in [25]), has only recently attracted the attention it deserves, but Smyth is rarely cited (e.g., [26]).

Smyth was a lateral thinker and developed hypothetical models for how genes regulate differentiation and developmental shifts in *Echinococcus* based on the Jacob–Monod model of gene action (Figure 1) [7]. He went further, and based on his observations in tapeworms, developed a hypothetical ‘control circuit’ appropriate to the life cycles of parasites in general, again based on the Jacob–Monod model of gene action (Figure 2). Such models should complement the search for valuable targets that can compromise parasite development from the ‘omics’ data now available.

The future

Francois Jacob died earlier this year, and in an obituary, Michel Morange [27] said ‘His death marks the end of a golden age of biology, in which members of a relatively small international community were free to pursue whatever question they wanted, with the possibility that they would make huge strides in discovery’. Desmond Smyth was only one parasitologist of his era who did likewise, and therefore let us not forget and build on his, and that of his colleagues, forward thinking. Those of us who have been mentored by and/or worked with seminal thinkers such as Desmond Smyth have an obligation to remind the parasitological community of their contributions as new technologies provide the vehicle for their hypotheses to be tested.

Box 1. *Echinococcus*: impact of *in vitro* cultivation studies

Species of *Echinococcus* are taeniid cestodes with a two-host life cycle, comprising the adult sexual stage in the small intestine of a carnivore definitive host and a larval cystic stage in the tissues of a herbivore or omnivore intermediate host. The cyst supports the development of protoscoleces, which if ingested by the correct definitive host will develop into the adult tapeworm.

Studies on the *in vitro* cultivation of the different stages in the life cycle have demonstrated:

- The conditions that will support growth and development of the larval and adult stages *in vitro*.
- The importance of a contact stimulus for adult development – this led to the discovery of a rostellar gland in the adult cestode.
- Pronounced developmental plasticity of *Echinococcus* considered unique in metazoans; for example, larval protoscolex has the potential to develop into another larval cyst or an adult tapeworm, and under adverse conditions adult worms can become cystic.
- The influence of environmental factors in governing the direction of development.
- A complex, sophisticated process of cytodifferentiation.
- Bile as a determinant governing host specificity in the definitive host.
- The parasite origin of the unique laminated layer that characterizes the larval ‘hydatid’ cyst.
- Radical shifts in our understanding of host specificity based on comparative studies of *Echinococcus* from different hosts. Laid the foundation for a better understanding of the epidemiology of

Echinococcus infections and supported a complete revision of the taxonomy of *Echinococcus* reflecting the observations and nomenclature of early taxonomists.

- Proposed the multipotential stem cell-like nature of germinal cells in species of *Echinococcus*.
- A unique degree of heterogeneous morphogenesis making *Echinococcus* spp. excellent models for differentiation studies in invertebrates and vertebrates.

Resulting questions now waiting to be addressed:

- What are the fundamental metabolic changes that take place in the transition from protoscolex to adult worm?
- How does the environment affect the parasite and what are the shifts in metabolism which occur between different hosts?
- Is it possible to identify the environmental cues/switches that regulate development, and could these be potential targets for chemotherapeutic attack?
- Why will a particular species of *Echinococcus* develop in one host and not another?
- What factors govern infectivity to humans?
- Can knowledge of the molecular basis of host susceptibility be used as a novel control strategy?
- What is the nature and functional significance of the rostellar gland secretions?
- What molecular interactions occur at the definitive host–parasite interface?

Acknowledgments

With thanks to Mark Preston for redrawing the figures so carefully.

References

- 1 Berriman, M. *et al.* (2009) The genome of the blood fluke *Schistosoma mansoni*. *Nature* 460, 352–358
- 2 Jex, A.R. *et al.* (2011) *Ascaris suum* draft genome. *Nature* 479, 529–533
- 3 Young, N.D. *et al.* (2012) Whole-genome sequence of *Schistosoma haematobium*. *Nat. Genet.* 44, 221–225
- 4 Cui, S-J. *et al.* (2013) Proteomic characterization of larval and adult developmental stages in *Echinococcus granulosus* reveals novel insight into host–parasite interactions. *J. Proteomics* 84, 158–175
- 5 de Sousa, T.N. *et al.* (2013) ‘Omics’ in the study of the major parasitic diseases malaria and schistosomiasis. *Infect. Genet. Evol.* 19, 258–273
- 6 Tsai, J.I. *et al.* (2013) The genomes of four tapeworm species reveal adaptations to parasitism. *Nature* 496, 57–63
- 7 Smyth, J.D. *et al.* (1967) Factors controlling the differentiation of the hydatid organism, *Echinococcus granulosus*, into cystic or strobilar stages *in vitro*. *Nature* 211, 1374–1377
- 8 Smyth, J.D. (1969) Parasites as biological models. *Parasitology* 59, 73–91
- 9 Smyth, J.D. *et al.* (1969) Observations on nuclear secretions in the rostellar gland cells of *Echinococcus granulosus* (Cestoda). *Nucleus* 12, 47–56
- 10 Smyth, J.D. and Smyth, M.M. (1964) Natural and experimental hosts of *Echinococcus granulosus* and *E. multilocularis*, with comments on the genetics of speciation in the genus *Echinococcus*. *Parasitology* 54, 493–514
- 11 Smyth, J.D. (1967) Studies on tapeworm physiology. XI. *In vitro* cultivation of *Echinococcus granulosus* from the protoscolex to the strobilate stage. *Parasitology* 57, 111–133
- 12 Smyth, J.D. *et al.* (1967) Further analysis of the factors controlling strobilization, differentiation, and maturation of *Echinococcus granulosus in vitro*. *Exp. Parasitol.* 21, 31–41
- 13 Smyth, J.D. (1968) *In vitro* studies and host-specificity in *Echinococcus*. *Bull. World Health Organ.* 39, 5–12
- 14 Smyth, J.D. and Smyth, M.M. (1968) Some aspects of host specificity in *Echinococcus granulosus*. *Helminthologia* 9, 519–527
- 15 Smyth, J.D. (1971) Development of monozoic forms of *Echinococcus granulosus* during *in vitro* culture. *Int. J. Parasitol.* 1, 121–124
- 16 Smyth, J.D. (1972) Changes in the digestive surface of cestodes during larval/adult differentiation. In *Functional Aspects of Parasite Surfaces* (Taylor, A.E.R. and Muller, R., eds), Symposium of the British Society of Parasitology 10, pp. 41–70, Blackwell Scientific
- 17 Smyth, J.D. and Davies, Z. (1974) *In vitro* culture of the strobilar stage of *Echinococcus granulosus* (sheep strain): a review of basic problems and results. *Int. J. Parasitol.* 4, 631–644
- 18 Smyth, J.D. and Davies, Z. (1974) Occurrence of physiological strains of *Echinococcus granulosus* demonstrated by the *in vitro* culture of protoscoleces from sheep and horse hydatid cysts. *Int. J. Parasitol.* 4, 443–445
- 19 Smyth, J.D. and Davies, Z. (1975) *In vitro* suppression of segmentation in *Echinococcus multilocularis* with morphological transformation of protoscoleces into monozoic adults. *Parasitology* 71, 125–135
- 20 Smyth, J.D. and Barrett, N.J. (1979) *Echinococcus multilocularis*: further observations on strobilar differentiation *in vitro*. *Rev. Iberica Parasitol.* 39, 39–53
- 21 Smyth, J.D. (1982) Speciation in *Echinococcus*: biological and biochemical criteria. *Rev. Iberica Parasitol.* 25–34 Special Volume
- 22 Sommer, R.J. (2009) The future of evo-devo: model systems and evolutionary theory. *Nat. Rev. Genet.* 10, 416–422
- 23 Gustafsson, M.K.S. (1976) Basic cell types in *Echinococcus granulosus* (Cestoda, Cyclophylidae). *Acta Zool. Fennica* 146, 1–16
- 24 Smyth, J.D. *et al.* (1987) Asexual and sexual differentiation in cestodes: especially *Mesocostoides* and *Echinococcus*. In *Molecular Paradigms for Eradicating Helminth Parasites* (MacInnis, A.J., ed.), pp. 19–33, Alan R. Liss
- 25 Thompson, R.C.A. (1995) Biology and systematics of *Echinococcus*. In *Echinococcus and Hydatid Disease* (Thompson, R.C.A. and Lymbery, A.J., eds), pp. 1–50, CAB International
- 26 Brehm, K. (2010) *Echinococcus multilocularis* as an experimental model in stem cell research and molecular host–parasite interaction. *Parasitology* 137, 537–555
- 27 Morange, M. (2013) Francois Jacob (1920–2013). *Nature* 497, 440